PREPARATION AND CHARACTERIZATION OF NOVEL GELATIN AND CARRAGEENAN BASED HYDROGELS FOR TOPICAL DELIVERY

Thesis submitted to
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BY:
JYOSTNA RANI PADHI
ROLL NO.- 413LS2043

Under the guidance of
Dr. BISMITA NAYAK

DEPARTMENT OF LIFE SCIENCE
NATIONAL INSTITUTE OF TECHNOLOGY,
ROURKELA -769008

राष्ट्रीय प्रौद्योगिकी संस्थान
NATIONAL INSTITUTE OF TECHNOLOGY
राउरकेला ROURKELA - 769008, ओडिशा ODISHA

RIGHT TO INFORMATION

Dr. Bismita Nayak, Ph.D.,
Assistant Professor
Department of Life Science
National Institute Of Technology Rourkela
Orissa, 769008
Email: nayakb@nitrkl.ac.in, bismita.nayak@gmail.com
Phone no.: 0661-2462682

Place: ...N.I.T. Rourkela...
Date: ...07/05/2015

CERTIFICATE

This is to certify that the thesis entitled "***Preparation and characterization of novel Gelatin and carrageenan based hydrogels for tropical drug delivery***" Submitted to National Institute of Technology, Rourkela for the partial fulfillment of the Master degree in Life science is a faithful record of bonafide and original research work carried out by **Mrs. Jyostna Rani Padhi** under my supervision and guidance. The results embodied in this thesis are new and have not been submitted to any other university or institution for award of any degree or diploma.

Bismita Nayak

Dr. Bismita Nayak
Bismita Nayak
Assistant Professor
Department of Life Science
NATIONAL INSTITUTE OF TECHNOLOGY
Rourkela-769008, Odisha, India

DECLARATION

I hereby declare that, the thesis entitled **"Preparation and characterization of novel gelatin and carrageenan based hydrogels for topical delivery***"*, submitted to the Department of Life Science, National Institute of Technology, Rourkela for the partial fulfillment of the Master Degree in Life Science, is a faithful record of bonafide research work carried out by me under the guidance and supervision of Dr. Bismita Nayak, Assistant Professor, Department of Life Science, National Institute of Technology, Rourkela. No part of this thesis has been submitted by any other research persons or any students.

Date – 11-05-15

Place- Rourkela

Jyostna Rani Padhi

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Place: Rourkela Jyostna Rani Padhi

Date:

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ABSTRACT

The hydrogels are 3D hydrophilic networks of homopolymeric or heteropolymeric chains which were first synthesized by Wichterle and Lim in 1954. The cross-linked hydrogels have the capability of absorbing huge amount of water without itself dissolving in it. The capacity to store water, softness and smartness make hydrogel a unique and ideal material. Hydrogels were prepared using gelatin and carrageenan in this work. The model drug used was ciprofloxacin. And the various characterizations like bright field microscopy, swelling profile, drug release studies and antimicrobial studies were done.

CHAPTER – 1

INTRODUCTION

The hydrogels are 3D hydrophilic networks of homopolymeric or heteropolymeric chains which were first synthesized by Wichterle and Lim in 1954 [1]. The cross-linked hydrogels have the capability of absorbing huge amount of water without itself dissolving in it. The capacity to store water, softness and smartness make hydrogel a unique and ideal material [2,3]. The hydrophilic functional groups that are adhered to the backbone of the polymer enable the hydrogels to absorb water whereas the cross-links between the network chains provides them with the property of resistance to disintegration [4]. The solute molecules can freely move inside the hydrogel due to the presence of water in it, and the polymer aids as a source/model to hold water together. The network of chains is interconnected to each other in the single polymer molecule of the hydrogel to form one big molecule on macroscopic scale [5]. Therefore, the hydrogels are hydrophilic gels that have the networks of polymeric chains which are found in the colloidal gels in which water is in the dispersion medium. It is a polymeric material that has the ability to swell and retain a significant amount of water within its structure without itself dissolving in it [6].

The hydrogel is present in a semi-solid state which gives rise to an interesting relaxation behavior which is not found in either a pure solid or a pure liquid. From the mechanical viewpoint, the hydrogels are characterized by an elastic modulus which exhibits a pronounced plateau extending to times at least of the order of seconds, and by a viscous modulus which is considerably smaller than the elastic modulus in the plateau region [5,7]. In response to the specific external stimuli such as the pH, electric field, temperature, etc., the hydrogel show a severe change in its volume. [8,9].

In the past 50 years hydrogels have been applied to wide range of applications like food additives [10], biomedical implants [11], pharmaceutical [12], diagnostics [13],tissue engineering and

regenerative medicines [14], separation of biomolecules or cells [15] and barrier materials to regulate biological adhesions [16], cellular immobility[17], biosensor and BioMEMs devices and drug carriers [18]. They have been serving as an ideal material for tissue engineering scaffolds due to their architectural analogy to the extracellular matrix (ECM) and presence of high water. And because of the property of having large amount of water, they are highly flexible to the natural tissue [19]. They have the ability to imbibe water upto 20 times more than its original molecular weight [20]. The best examples of hydrogels are the contact lenses [21], superabsorbants [22-24] and wound dressings [25, 26].

The use of hydrogel has various advantages. It is highly biocompatible, it is easy to modify according to their uses, it can be injected, and it can be used for the timed release of the nutrients and growth factors to ensure the proper growth of the tissue [27]. The polymers itself cannot form a single matrix of networks between themselves. In order to form the entanglement of chains between the polymers certain crosslinking agents are used. The crosslinking agents used can be basically divided into two types – chemical crosslinkers and physical crosslinkers [28]. The chemical crosslinkers applied in the hydrogels give rise to the covalent bonds among the different polymer chains. And the physical crosslinkers provide physical interactions among the polymers. Both the crosslinkers provide 3D integrity to the gels even in their swollen state. The hydrophilic linear polymer chains are capable of dissolving in water in the absence of the crosslinking points. This is due to the thermodynamic compatibility of water and the polymer chains. Whereas, the presence of the crosslinking points counter-balances the solubility by the retractive force of elasticity that are induced by the crosslinking points present in the network [29].

Many biodegradable polymers like chitosan, PMMA, PEEK, PVA etc. have been used for making hydrogels. These natural polymers have certain inherent characteristics that have made various research scholars to synthesize hydrogels for their utilization in various applications.

In this work we have chosen the natural polymers gelatin and i-carrageenan for procreating the hydrogel. Choosing gelatin as an alternative to other polymers was due to its inherent properties of being non-toxic, non-immunogenic, biodegradability and most importantly, reasonable price. It has a very eminent potential so as to be used with a range of medicinal agents [30]. It has been used as hemostatic and wound healing agent [31-33], wound dressings [34-37], as sealant for vascular prosthesis [38, 39] and in drug delivery systems such as hard and soft capsules [40, 41]. The benefit of using gelatin over collagen involves its inexpensiveness and high dissolving capability in water. The gels formed from gelatin are naturally biodegradable and show non-cytotoxicity toward human cells [42, 43]. In the present study, we have developed a gelatin-based hydrogel along with i-carrageenan. It has been reported that the carrageenans have high gelling abilities due to which they can be used for the controlled release of drug. When used in smaller concentrations, it doesn't induce a toxic reaction. On cooling, i-carrageenan undergoes coil-helix conformational transitions which lead to gelation. Gelation of i-carrageenan is enhanced mainly by calcium ions and forms soft elastic gels [44]. It has been reported that i-carrageenan has the maximum activity of preventing the aggregation that are induced by ADP or adrenaline [45]. These carrageenans have distinct biological activities. They can act as an anticoagulant, have antiviral, antitumor and anti-HIV effects, can behave as immunomodulatory and antithrombic agents [44].

The main aim of our work is to develop an efficient drug delivery system by using the above mentioned polymers and Ciprofloxacin is incorporated within its matrix as the drug. The drugs

that we take in do not reach the target sites and finally are metabolized out through our body. So hydrogels loaded with drug can be used for the timed release of the drug so that the drug is able to reach the targeted site.

CHAPTER - 2

REVIEW OF LITERATURE

The hydrogels are 3D hydrophilic networks of homopolymeric or heteropolymeric chains which were first synthesized by Wichterle and Lim in 1954 [1]. The term hydrogel is being used by the biomaterial scientists to describe the polymeric cross-linked network structures [46]. The hydrogels are a class of biomaterials that are obtained from a group of synthetic polymers and natural polymers. They have the ability of absorbing and retaining significant amount of water [47]. The structure of hydrogel is created by a group of hydrophilic molecules (that exist in a polymeric network) upon the hydration in an aqueous environment. The cross-linked hydrogels have the capability of absorbing huge amount of water without itself dissolving in it. This is because of the crosslinkers that are used in it. And these crosslinkers facilitate insolubility in water due to the presence of hydrogen bonding and ionic interaction. And they also provide the required physical integrity and mechanical strength to the hydrogels. The capacity to store water, softness and smartness make hydrogel a unique and ideal material [2,3].

2.1 Mechanism of network formation

The linking of macromolecular chains together leads to the formation of progressively larger branched soluble polymers and is referred to as gelation. Gelation depends on the conformation and structure of the preliminary material. The combination of such poly-dispersed soluble branched polymer is called 'sol'. Extension of the linking process results in decreased solubility with the increase in the size of the branched polymer. This 'infinite polymer' is called the 'gel' or 'network' and is permeated with finite branched polymers. Gelation is defined as the transition of a system with infinite molecules from a system with predetermined branched polymer. It can also be termed as 'sol-gel transition'. The critical point where there is the appearance of first gel appears is called the 'gel point' [48, 49]. Different types of gelation mechanism are summarized in Figure 1. The process of gelation takes place either by chemical

linking or by physical linking. Physical gels can be further classified as weak gels and strong physical gels. The weak physical gels contain the links that are reversible as they are formed from temporary links between chains. These links have the capability of continuously breaking and reforming, and they have finite lifetimes. Examples of weak physical bonds are ionic associations, hydrogen bond, and block copolymer micelles. The strong physical gel contains strong physical bonds between the polymer chains and is efficiently permanent at a given set of experimental conditions. Therefore, strong physical gels are similar to chemical gels. Examples include glassy nodules and lamellar microcrystals [49].

On the other hand, chemical gelation involves the formation of the covalent bonds between the polymer molecules and always results in a strong gel. The three main processes that are used for chemical gelation include addition polymerization, condensation, and vulcanization [49].

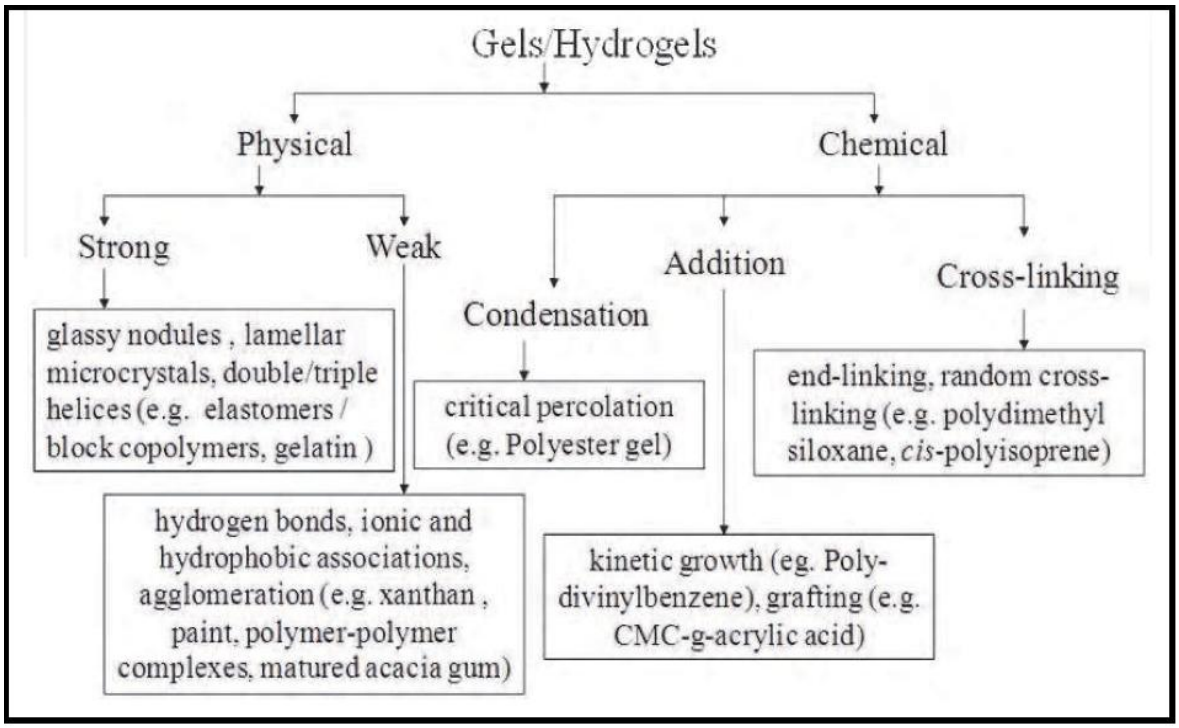

Figure 1 - Classification of gelation mechanisms with relevant examples. (Adapted from *Hydrogels: Methods of Preparation, Characterisation and Applications*)

2.2 Classification of hydrogels -

Depending upon the sources, various preparation methods, ionic charges, the nature of crosslinking or rate of biodegradation, and nature of swelling according to the changes in the environment, hydrogels have been classified in many ways. A comprehensive classification of hydrogels is shown in Figure 2 [50, 51, 52]. But among all, one of the most important classifications is based on their crosslinking nature (Figure 2). The stability of hydrogels, as a system, in their swollen condition is due to the presence of either physical or chemical crosslinking. The hydrogels that are chemically crosslinked are also known as **thermosetting hydrogels.** They also called as **permanent gels**. They are not soluble in any solvents unless there is cleavage of the covalently crosslinked points. Furthermore, they cannot be remoulded through melting, in heat. They can be made by using any of these two methods, viz, (a) Copolymerizing hydrophilic monomers with crosslinkers, and (b) Crosslinking of water-soluble polymer segments with irradiation method (microwave, UV, gamma-irradiation and electron beam) or with di and/or multi functional crosslinkers.

The value of hydrogels that are chemically crosslinked is frequently limited by the lack of post-process modifications and processibility. Due to this, the shaping of hydrogel is carried out along with the reaction step of polymerization. Besides the crosslinking agents are highly toxic and the residues are needed to be completely removed before they are used as biomaterials [53].

On the other hand, physically crosslinked hydrogels, have the capability of maintaining their physical stability because of the presence of reversible physical junction domains that are associated with hydrophobic interaction, chain entanglements, crystallinity, hydrogen bonding, and/or ionic complexation [54,55,56]. The hydrogels that are physically crosslinked, are also known as **temporary gels** or **thermoplastic hydrogels**. The swelling of the physically crosslinked hydrogels is typically dependent on the thermodynamic parameters such as pH,

temperature, ionic strength and/or salt type. Variations in these parameters may decrease or increase their swelling. The preparation of these hydrogels avoids the use of toxic crosslinkers. [57].

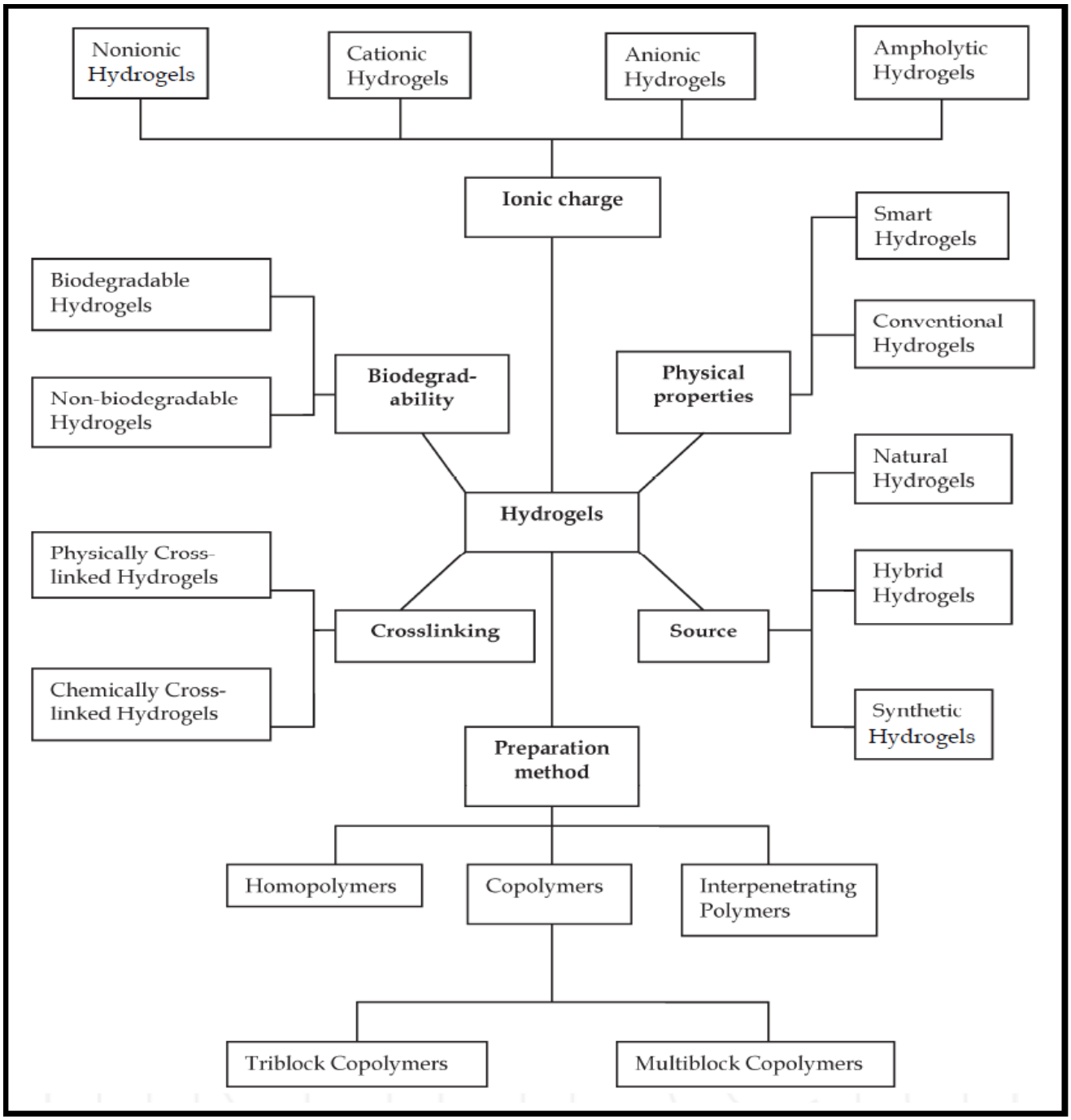

Figure 2 - Classification of Hydrogels (Adapted from *Hydrogel Biomaterials*)

2.2.1. Classification based on source :

Hydrogels can be divided into three groups based on the materials used for their synthesis. They are as follows-

(a) **Natural hydrogels** are being prepared by using natural polymers. They have the advantage of being biocompatible and biodegradable and they support the cellular activities. But their main disadvantage is that they do not have sufficient mechanical properties. They may contain pathogen also. And they have the capability of evoking immune and inflammatory responses. Examples include proteins like collagen and gelatin, and polysaccharides like alginate and agarose [58].

(b) **Hybrid hydrogels** are prepared by using nanoparticles alongwith different polymers. They are highly hydrated polymeric networks that are either covalently or physically crosslinked with each other and/or with nanostructures or nanoparticles. They have the advantage of mimicking the microenvironment and structure of native tissue due to the presence of the interconnected porous structure. A broad range of nanoparticles, such as polymeric, ceramic, carbon-based, and metallic nanomaterials are incorporated within the structure of hydrogel to obtain nanocomposites with tailored functionality. They are also called as Nanocomposite hydrogels. They can be engineered to possess superior chemical, physical, electrical, and biological properties [59].

(c) **Synthetic hydrogels**(polymer) are prepared by chemical polymerization. The major advantage of it is, the inherent bioactive properties are absent in it and they possess sufficient mechanical properties. Examples include acrylic acid, vinyl acetate, hydroxyethylmethacrylate, and methacrylic acid [58].

2.2.2. Classification based on polymeric composition:

The preparation method leads to the formation of some important classes of hydrogels. They can be classified as follows:

(a) **Homopolymeric hydrogels** are referred to as the polymeric network that is derived from a single monomer species, which is the basic structural unit for any polymer network. Depending on the technique of polymerization and the nature of the monomer, homopolymers may contain the cross-linked skeletal structure [6].

(b) **Copolymeric hydrogels** are made up of more than two different species of monomer having at least one hydrophilic component, that are arranged in a random, block or alternating configuration along the chain of the polymer network [6].

(c) **Multipolymer Interpenetrating polymeric hydrogel (IPN)** is made of two natural polymer component and/or cross-linked synthetic component, which are independent and are contained in a network form. It is an important category of hydrogels. In semi-IPN hydrogel, one component is non-cross-linked polymer and other component is cross-linked polymer [6].

2.2.3. Classification based on configuration

The classification of hydrogels depending on their chemical composition and physical structure is as follows:

(a) **Amorphous** (non-crystalline) hydrogels are free-flowing (random) gels that are packaged in tubes, foil packets, and spray bottles which are used in wound dressings.

(b) **Semicrystalline** hydrogel is a complex mixture of amorphous and crystalline phases.

(c) **Crystalline** hydrogels are not free-flowing gels and have compact structure.

2.2.4. Classification based on different types of cross-linking:

The hydrogels can be broadly divided into two categories based on the physical or chemical nature of the cross-linked junctions.

(a) **Chemically cross-linked** hydrogels have networks with permanent junctions [6].

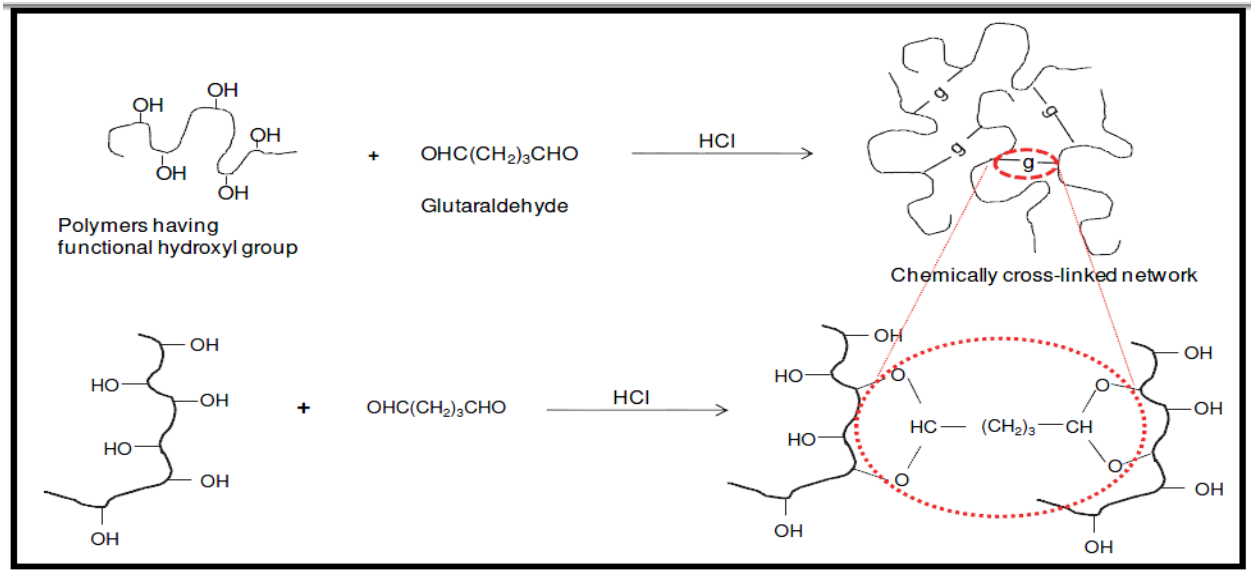

Figure 3 – Showing illustration of the use of chemical cross-linker to obtain a network of cross-linked hydrogel (Adapted from *Hydrogels: Methods of Preparation, Characterisation and Applications*)

(b) **Physically cross-linked networks** have temporary junctions that arise from either physical interactions such as hydrogen bonds, ionic interactions, and hydrophobic interactions, or entanglements of polymer chain [6].

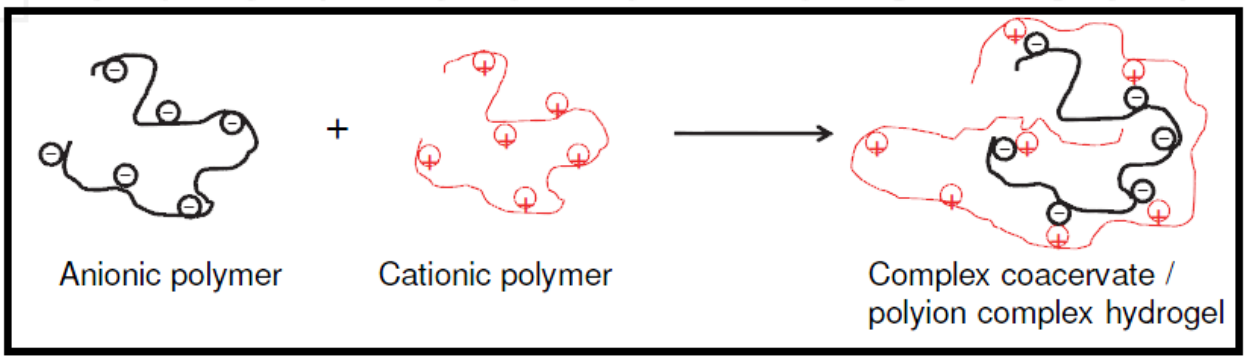

Figure 4 – Showing the mechanism of physical crosslinking between anionic polymer and cationic polymer. (Adapted from *Hydrogels: Methods of Preparation, Characterisation and Applications*)

2.2.5. Classification based on physical appearance:

Hydrogels can appear as film, matrix, or microsphere. These appearances depend on the technique of polymerization that is involved in the process of preparation.

2.2.6. Classification according to electrical charge in the network:

Hydrogels can be classified into four groups on the basis of absence or presence of electrical charge located on the crosslinked chains:

(a) **Nonionic** (neutral) such as dextran.

(b) **Ionic** which includes both anionic (such as carrageenan) and cationic (such as chitosan).

(c) **Ampholytic** contains both basic and acidic groups such as collagen.

(d) **Zwitterionic** (polybetaines) contains both cationic and anionic groups in each structural repeating unit [6].

2.2.7. Classification according to their biodegradability :

Hydrogels can be classified into two groups on basis of their solubility in the water and enzymes.

(a) **Biodegradable** hydrogels have the capability of breaking down to simpler molecules, inside the body, with both water and enzymes.

(b) **Non-biodegradable** hydrogels are not broken down by the water and enzymes [53].

2.2.8. Classification according to their physical properties :

Hydrogels can be classified into two groups on basis of their responses according to the changes in environment.

(a) **Smart** hydrogels are also known as stimulus-responsive polymeric hydrogels. Smart hydrogel is defined as the polymer network able to respond to external stimuli through abrupt changes in the physical nature of the network. They respond with sharp, large property changes in response to small change in physical or chemical conditions. Types

of stimuli include pH, temperature, ionic strength, solvent composition, pressure, electrical potential, radiation and chemical and biological agents. Common stimuli for smart hydrogels in biological applications are pH, temperature and ionic strength [60]. Figure 3 shows the different types of smart hydrogels.

(b) **Conventional** hydrogels are made by the crosslinking of the polymers and they do not show any response according to the change in environment.

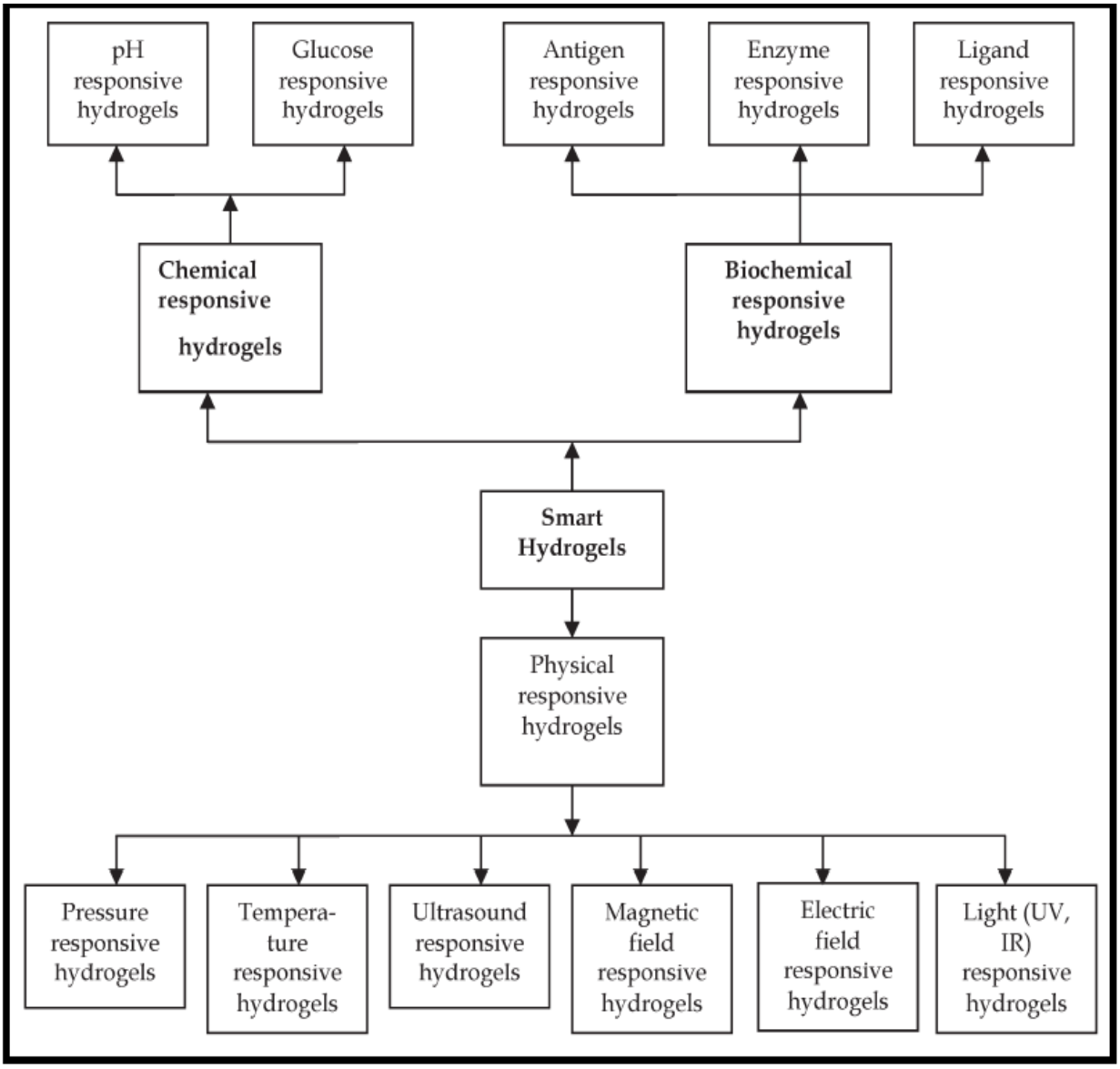

Figure 5 - Types of Smart Hydrogels (Adapted from *Hydrogel Biomaterials*)

2.2.8. Classification according to the presence or absence of water :

(a) **Organogel** are semi-solid systems, in which an organic liquid phase is immobilized by a three-dimensional network composed of self-assembled, intertwined gelator fibers. It is a type of hydrogel that has non-crystalline, thermoplastic solid material which is composed of a liquid organic phase entangled in a 3D cross-linked network. The liquid can be either a mineral oil, an organic solvent, or a vegetable oil. Organogels are used in a number of applications, such as in cosmetics, pharmaceuticals, and food [61].

(b) **Xerogel** is a dry from of a hydrogel. It is a solid that is formed from a hydrogel by drying it with unobstructed shrinkage. They are highly porous (15–50%) in nature along with tiny pore sizes (1–10 nm). They have enormous surface area (150–900 m^2/g). [49, 62].

(c) **Aerogel** is derived from a gel by the technique of supercritical drying. Here the liquid part of the gel is replaced with a gas. It is effective as a thermal insulator and has extremely low density. It is also called solid smoke, frozen smoke, or blue smoke due to its nature of translucence and the approach of light scattering in the material. Examples include silicon and carbon aerogels which are used in buildings double window [49, 62].

(d) **Oleogels** are defined as lipophilic liquid and solid mixtures, in which solid lipid materials (oleogelators) with lower concentrations (<10 wt.%) entrap bulk liquid oil by the ways of the formation of network of oleogelators in the bulk oil [63].

(e) **Bigel** are defined as oleogel and hydrogel mixtures [64].

2.3 Applications of hydrogels :

The unique properties of hydrogels, for their applications in various fields, include:

- Soft and tissue-like physical properties,

- Good oxygen permeability,
- Superior biocompatibility,
- Low protein adsorption,
- Upon implantation has minimum frictional irritation within the surrounding tissues,
- Micro-porous structure for additional transport channels,
- Easy for surface modification with specific biomolecules, and
- Can be injected as a solution that gels at body temperature.

These features of hydrogels has made them ideal biomaterials for applications in cell encapsulation, drug delivery system, contact lenses, scaffolds for tissue engineering, biosensors, soft tissue replacement, intelligent cell culture substrates, wound dressing, and many more.

2.3.1. Hydrogels for cell encapsulation

Technology of cell encapsulation is a promising therapeutic mode for diabetes, cancer, hemophilia, and renal failure [65, 66]. The selection of the most appropriate biomaterial as a membrane for encapsulating the cells is a challenge towards the success of the therapy of cell encapsulation. Some properties like microporous structure, biocompatibility, and minimum surface irritation with the surrounding tissues of hydrogels has drawn attention towards themselves for this application. The hydrogels can be designed with required porosity that resists the entrance of immune cells and allows transfer of oxygen, nutrients, stimuli, and/or waste through the pores. The genetically modified alginates [67] and polyethylene oxide based hydrogels [68] have been extensively studied as cell encapsulation systems.

2.3.2. Hydrogels for drug delivery applications

The drug delivery systems that are well-designed must control the release of solute over time. Many biomaterials have been scrutinized to control release of drug. But the hydrogels show two

distinct advantages. (i) Easy diffusion of the drugs through the hydrogels where the rate of drug release can be monitored by changing the density of crosslinking. (ii) The hydrogels may interact less strongly with drugs as compared with hydrophobic materials [69].

The hydrogels are having some useful applications in pharmaceutical sciences and drug delivery because of their large amount of water content. The hydrogel-based drug delivery system can be applied in various fields like ocular, oral, conventional, epidermal and subcutaneous application. They are also applied in the delivery of gene and proteins.

2.3.3. Hydrogels for tissue engineering scaffolds

Scaffolds are mainly 3D structural templates that support cell migration, cell adhesion, cell proliferation, cell differentiation, and provide guidance for formation of new tissue. The scaffold material that is chosen should be reproducible and biocompatible, with high porosity and well-organized inter-connectivity [70].

Hydrogels have emerged as useful scaffolding biomaterials as they mimic the natural tissues of the body. They are biocompatible and porous for diffusion for nutrient and waste. Both natural and synthetic hydrogels have been used as scaffolds in tissue engineering, in order to repair tendon, ligament, cartilage, skin, blood vessels and heart valves [70, 71]. The synthetic hydrogels that have been focused as scaffolds are poly(ethylene oxide) (PEO), polyurethanes (PU), poly(vinyl alcohol) (PVA), poly(*N*isopropylacrylamide) (PNIPAAm), and poly(acrylic acid) (PAA), whereas, naturally derived hydrogels are agarose, chitosan, fibrin, collagen, alginate, hyaluronic acid (HA), and gelatin [72].

2.3.4. Hydrogels for contact lens application

The synthetic hydrogels are suitable in the applications of contact lens when the refractive power of cornea is compromised. In adjunct to their softness and biocompatibility, the inter-connected

microstructures of hydrogels help in the diffusion of oxygen to the epithelial layer of the cornea. Some hydrogels have good transparency, high refractive index, and modulus which are essential for this product. Poly (HEMA) was the first hydrogel that has been used as a contact lens in 1960 [1]. Since all these required features cannot be derived from single hydrophilic polymer structure, therefore copolymers have been developed from a group of hydrophilic monomers like methacrylic acid (MAA), dimethylacrylamide (DMAAm), and N-vinyl pyrrolidone (NVP) and hydrophobic monomers like methyl methacrylate (MMA), perfluoro polyethers (PFPE), and silicon-containing monomers that are used to design contact lenses [73].

2.3.5. Hydrogels for Biomedical engineering and biomaterial

Due to its biodegradability and biocompatibility, the hydrogels have been efficiently and effectively used in various biomedical applications. For their biomedical applications, the polymers used have been tested for cytotoxicity and in-vivo toxicity. The utilization of hydrogels in the area of biomedical engineering includes formation of phospholipid bilayer, system for energy conversion, as a component of EEG and ECG medical electrodes, and transport of mass. And because of their bioadhesiveness they have been used in wound dressings and as superabsorbent biomaterials.

2.3.6. Hydrogels for Agricultural applications

They have been also used in various applications of agriculture. The addition of the hydrogels to the surface of the soil increased the water holding capacity of the soil and minimizes the loss of nutrients from the soil. But they show low efficiency in the saline soil. The hydrogels are applied to the soil directly or by spraying.

Besides the above applications the hydrogels have also been applied to a wide range of applications like food additives [10], biomedical implants [11], pharmaceutical [12], diagnostics

[13], tissue engineering and regenerative medicines [14], separation of biomolecules or cells [15], and barrier materials to regulate biological adhesions [16], cellular immobility [17], biosensor and BioMEMs devices and drug carriers [18].

2.4. Limitations

Besides of having the various unique properties the hydrogels have some generalized limitations. The limitations are as follows:

- Difficult to load with drugs/nutrients,
- May be difficult to sterilize,
- Non-adherent,
- Can be hard to handle,
- Lack of mechanical strength when worked for as scaffolds for tissue engineering purposes,
- High cost, and
- Because of low tensile potency many hydrogels limit their use in load bearing application and result in flowing away from the targeted local site [74, 75].

2.5. Gelatin:

Gelatin is a protein that is derived from partial hydrolysis of collagen from the bone, skin, and connective tissue of the animals like bovine, porcine, and fish. The typical structure of gelatin is: Ala-Gly-Pro-Arg-Gly-Glu-4Hyp-Gly-Pro-. It is an easily digestible protein that contains all the essential amino acids except tryptophan [77]. Choosing gelatin as an alternative to other polymers was due to its inherent properties of being non-toxic, non-immunogenic, biodegradability and most importantly, reasonable price. It has a very eminent potential so as to

be used with a range of medicinal agents [30]. It has been used as hemostatic and wound healing agent [31-33], wound dressings [34-37], as sealant for vascular prosthesis [38, 39] and in drug delivery systems such as hard and soft capsules [40, 41]. The benefit of using gelatin over collagen involves its inexpensiveness and high dissolving capability in water. The gels formed from gelatin are naturally biodegradable and show non-cytotoxicity toward human cells [42, 43].

2.5.1 **Properties of gelatin -**

Gelatin has some unique properties due to which it has been widely used in different fields. The properties are:

- It has high gelling capability due to the presence of proline and hydroxyproline residues.
- It is highly viscoelastic in nature which is revealed by rheological testing.
- It is inherently biocompatible in nature and therefore has been widely used for the development of hydrogels for various biomedical applications, including controlled delivery systems.
- It is thermostable in nature as it contains a large number of glycine, proline and 4-hydroxyproline residues. Its thermostability is associated with basic mechanism of gelatin which is related to the reverse coil-to-helix transitions. The transition is triggered by cooling solutions below 30°C, during which the helices that are created, are similar to the collagen triple-helix. The gelation process for gelatin is thermo-reversible, which means that gelatin gels melt by raising the temperature.

- It is inexpensive and biodegradable due to which it is attractive for forming hydrogel packaging.
- It is amphoteric in nature, because of which it provides less dipole moment though they contain various charged, polar and non-polar amino acid residues.
- It is highly biocompatible and biodegradable in the physiological environment and therefore has been used as a drug carrier.
- Its structure facilitates multiple combinations of molecular interactions in the formation of hydrogels.
- It has a unique property of melting in mouth due to which it is used in water-gel desserts.

2.5.2. Uses of gelatin:

Due to its viscoelastic properties and gel-forming capabilities, gelatin has been used in various fields like food, photography, cosmetics and pharmaceuticals. In food industry it is used as a fining agent, emulsifier, foaming agent, colloid stabilizers, biodegradable packaging materials and micro-encapsulating agents. At present, the use of natural polyampholytic hydrogels in applications such as gelatin-casings, with the ability to absorb large quantities of water are becoming more important in the field of medicine, pharmacy, agriculture and biodegradable food packaging. It is commonly used in food as a gelling agent, photography, pharmaceuticals, and cosmetic manufacturing. It has been widely used for various pharmaceutical and biomedical applications. Its other uses include:

- It is used to culture adherent cells.
- It may be used in teas, soups or brews by those who are tannin-sensitive.
- Gelatin is used to make the pharmaceutical capsules.

- Gelatin is used in medical devices that are implantable, such as, in some bone void fillers.
- Gelatin is used in nail polish remover.
- In the food industries, gelatin is used for its texture, chewiness, foam stabilization, creaminess, emulsification, gelling, and water binding capacity.
- In the pharmaceutical and medical engineering, gelatin is used as a matrix for implants, in injectable drug delivery.
- Gelatin is also prominently used as a stabilizer in live attenuated vaccines for the immunization against measles, mumps, diphtheria, rabies, and tetanus toxin.
- Gelatin-based constructs are mucoadhesive in nature and therefore are often employed as mucoadhesive delivery systems [78, 79].

2.6. i-Carrageenan:

i-Carrageenan is a natural polysaccharide that is obtained from edible red seaweeds of the class Rhodophyceae. The name "Carrageenan" is derived from the *Chondrus crispus* and *Eucheuma denticulatum* species of seaweed known as Carrageen Moss or Irish moss in England, and Carraigin in Ireland. It has been used gelatin and as a home remedy to cure coughs and colds. It is a sulphated, anionic, and linear polysaccharide that has high molecular weight. It is composed of the alternate units of d-galactose and 3,6-anhydro-galactose that are linked together by α-1,3 and β-1,4 glycosidic linkage. The regular repeating unit for (iota) ι-carrageenan is the disaccharide[→3)-α-D-galactose-6-sulfate-(1→4)-β-D -3,6-anhydrogalactose-2-sulfate-(l →] (stucture is shown below in figure 6) . There are different types of carrageenan which have been classified due to their primary differences in having the number and position of the presence of ester sulfate groups and the content of 3,6-anhydro-galactose. Iota type carrageenan has an ester

sulfate content of about 28 to 30% and a 3,6-AG content of about25 to 30%. When used in smaller concentrations, it doesn't induce a toxic reaction.

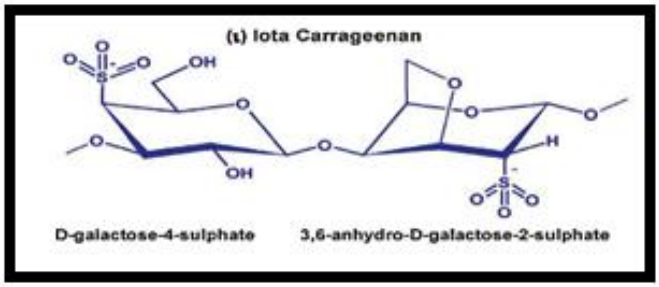

Figure- 6: Structure of Iota-Carrageenan (Adapted from *Carrageenan: A review*)

2.6.1. Properties of i-Carrageenan:

1) On cooling, i-carrageenan undergoes coil-helix conformational transitions which lead to gelation. Gelation of i-carrageenan is enhanced mainly by calcium ions and forms soft elastic gels [44].
2) It is highly viscous in nature and has high gel forming ability.
3) They act as an anticoagulant due to the presence of sulfate groups.
4) It forms right-handed helices while forming gel and calcium ion promotes gel formation in it.
5) Metabolism is by hydrolysis of glycosidic linkages at lower pH, especially pH $\leq$ 3.0, and also desulfation by sulfatases.
6) It has diverse biological activities which includes immunomodulatory, antithrombotic, antiviral and antitumor effects.
7) These negatively charged molecules exert their inhibitory effect by interacting with the positive charges on the virus or on the cell surface and thereby prevent the penetration of the virus into the host cells.
8) i-Carrageenan has been reported to have anti-HIV activity, but its strong anticoagulant activity is considered to be an adverse reaction when used as a therapeutic drug for AIDS.

2.6.2. Uses of i-carrageenan:

Since 400 AD they have been used as home remedies in United Kingdom. It is a vegetarian option against gelatin. Since then it has been used in wide variety of commercial applications. Its uses include:

1) As an gelling, thickening, stabilising agent, and an emulsifying agent in food products and sauces,
2) They are used in experimental medicine, pharmaceutical formulations in pharmaceutical applications and as experimental medicine this substance is often used for the testing of anti-inflammatory agents,
3) It has been used in cosmetics and industrial applications,
4) Desserts, ice cream, cream, milkshakes, salad dressings, sweetened condensed milks, and sauces: gel to increase viscosity
5) As an clarifier to remove haze-causing proteins in beer,
6) As an stabilizer to prevent constituents separating in toothpaste,
7) As an thickener in shampoo and cosmetic creams,
8) In biotechnology used as an gel to immobilize cells/enzymes, and
9) Used in Vegetarian hot dogs [44].

2.7. Glutaraldehyde:

Glutaraldehyde (GA) is an organic compound having chemical formula $CH_2(CH_2CHO)_2$. It is a pungent, colorless, and oily liquid. It is an aliphatic dialdehyde that undergoes most of the typical aldehyde reactions to form acetals, cyanohydrins, oximes, hydrazones and bisulfite complex. Glutaraldehyde is an excellent example of synthetic crosslinker. One of the advantages of using

glutaraldehyde is the presence of bifunctionality groups i.e. aldehydic and alcoholic group, due to which it forms crosslinks efficiently [80].

2.8. Ciprofloxacin Hydrochloride:

Ciprofloxacin hydrochloride is the monohydrochloride monohydrate salt of ciprofloxacin (1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid). It is a faintly yellowish to light yellow crystalline substance with a molecular weight of 385.8 g/mol. Its empirical formula is $C_{17}H_{18}FN_3O_3HCl•H_2O$. It is an antibiotic in a group of drugs called fluoroquinolones. It is a broad-spectrum antibiotic active against both Gram-positive and Gram-negative bacteria. It functions by inhibiting DNA gyrase, a type II topoisomerase, and topoisomerase IV, enzymes which is necessary to separate bacterial DNA, thereby inhibiting cell division. Its chemical structure is shown in figure 7.

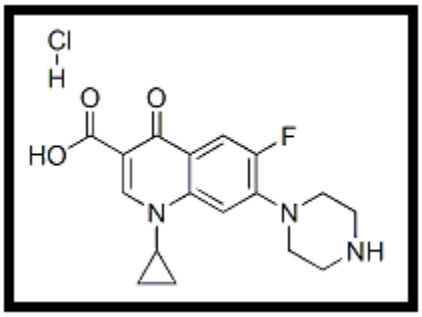

Figure 7: Structure of Ciprofloxacin Hydrochloride (Adapted from *web(81)*)

CHAPTER – 3

MATERIALS AND METHODS

3.1 Materials:

Gelatin, Irish moss (I-Carrageenan), Nutrient HiVeg Agar, Nutrient Broth were obtained from Himedia Laboratories Pvt. Ltd., Mumbai, India. Glutaraldehyde (50%) was obtained from Sigma-Aldrich, U.S.A., Hydrochloric acid (35%) was obtained from Merck Limited. All other chemicals were of analytical grade and used without further modification. All the microbial pathogens used in the study were obtained from Department of Pathology, Shri Ramachandra Bhanj Medical College, Cuttack, Odisha. All the experiments were carried out using double distilled water. Ciprofloxacin was procured from Fluka Biochemical, China. Goat blood and intestine were collected from local butcher shop. And throughout the study double distilled water (DW) was being used.

3.2 Preparation of formulations:

10% (w/w) of gelatin stock solution was prepared by dissolving the gelatin granules in the d/w at 50^0C with constant stirring at 600 rpm for 30 minutes. 1% (w/w) of i-carrageenan solution was prepared by dissolving the whitish powder of carrageenan in the warm d/w at 40^0C with constant stirring of 700 rpm for 30 minutes. 21ml of cross-linker was prepared by adding 10ml of glutaraldehyde with 10 ml of ethanol and 1 ml of 0.01N hydrochloric acid [79].

The optimization of the formation of gels was carried out by trial and error method. The two solutions were taken in different concentrations with increasing concentration of gelatin and decreasing concentration of i-carrageenan. The table is shown below:-

Sl. No.	Ratio of gelatin taken	Ratio of carrageenan taken	Sample code	Hydrogel formed
1	10	0	GCC1	Yes
2	9	1	GCC2	Yes
3	8	2	GCC3	Yes
4	7	3	GCC4	Yes
5	6	4	GCC5	Yes
6	5	5	GCC6	Yes

Both the mixtures were blended at 200 rpm on the magnetic stirrer, at 45°C for 20 minutes. The mixtures were admixed with the help of the prepared cross-linker. For 10 gms of the mixture 200µL of cross-linker was added for the proper formation of the hydrogel.

Gelatin (10% w/w) and carrageenan (1% w/w) stock solutions were freshly prepared. Both the stock solutions were maintained at 50°C. The gelatin and the carrageenan solutions were blended together at 200 rpm for 10 min and at varying proportions (table 1) followed by the addition of the cross-linking agent (0.5 ml of GA, 0.5ml of ethanol, 0.01ml of 0.1 N HCl). The mixture was stirred for 10 sec and immediately poured into the petri-plates/cylindrical moulds. Drug loaded hydrogels were prepared by dispersing 0.03 g of Ciprofloxacin (CF) in the gelatin solution. The gelatin solution containing CF was used for the preparation of the drug loaded hydrogels. The final concentration of the drug in the hydrogels was made upto 0.3% w/w.

3.3 Microscopy studies:

The microstructures of the uncrosslinked physical formulations were visualized under bright field microscope (LEICA-DM 750 equipped with ICC 50-HD camera, Germany). The formulations were converted into thin smears on the glass slides and were covered with glass cover-slips.

3.4 FTIR spectroscopy:

The prepared hydrogels were analyzed using FTIR spectrophotometer (Alpha-E, Bruker, USA). The spectrophotometer was being operated in the ATR mode. And the analysis was done in the wavenumber range of 4000 cm^{-1} to 500 cm^{-1}[79].

3.5 Mechanical Analysis:

The mechanical properties of the hydrogels were tested using static mechanical tester (Stable Microsystems, TA-HD plus, U.K.). For mechanical testing the hydrogels were prepared in cylindrical moulds [79].

3.6 Impedance analysis:

The electrical properties of the hydrogels were tested using an in-house built impedance analyser in the frequency range of 8 Hz- 8 KHz [79].

3.7 Cytocompatibility analysis:

The cytocompatibility of the hydrogels were determined using HaCaT and HEK293 cells. The cells were seeded in 96 well plates. 1 x 10^4 cells were added in each well. 20 µl of leachants (of hydrogels) was added in each well to understand the toxic effect of the leachants. The cell viability was determined using MTT assay.

3.8 Swelling studies:

The swelling profile of the hydrogel was determined at pH 7.4 (phosphate buffer). The weights of the hydrogels at an interval of 30 min for 7 h. the study was conducted at room temperature. The swelling index was calculated as per the following equation:

$$\text{Swelling Index (SI)} = (W_{final} - W_{initial})/ W_{initial}$$

Where, **W_{final} = Weight of sample after swelling at each time**, and **$W_{initial}$ = Weight of sample at the beginning, before the start of the study [79].**

3.9 Drug release studies:

The drug release studies were carried out using 0.5 gm of hydrogel samples. And the study was carried out by tube method using phosphate buffer of pH 7.4.The temperature of the PBS was maintained at 37°C. At regular intervals of time the PBS was replaced with fresh PBS for 12h. The replaced media was analyzed for the drug content using UV- visible spectrophotometer.

3.10 Antimicrobial test:

In order to corroborate the drug release studies, the antimicrobial test was performed using disc diffusion method. *E.coli* was used as the test microorganism. Hydrogel samples of 7mm diameter were used for the analysis. The antimicrobial activity was interrelated with the zone of inhibition of the microbial growth [79].

CHAPTER – 4

RESULTS

4.1. Photography:

Figure shows the photographs of the gels that are prepared in moulds.

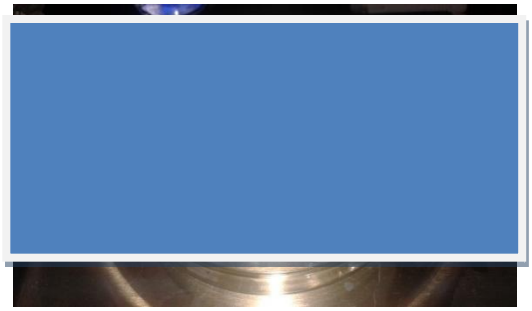

Figure 8 – showing the pictographs of prepared hydrogels.

4.2. Microscopy:

Microscopy depicts the formation of tiny circular structures.

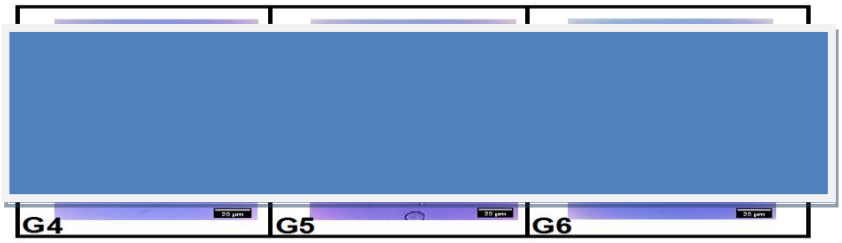

Figure 9 – showing the micrographs of prepared hydrogels.

4.3. FTIR spectroscopy:

FTIR is done to find the chemical interactions in the gels.

Figure 10 – showing the FTIR spectra of prepared hydrogels.

4.4. Mechanical Analysis:

It is done to find the cyclic compression, stress relaxation, and creep of the prepared hydrogels.

Figure 11 – showing the mechanical testing of prepared hydrogels.

4.5. Impedance analysis:

Figure 12 – showing the impedance testing of prepared hydrogels.

4.6. Cytocompatibility analysis:

This study shows the biocompatibility of the prepared samples.

Figure 13 – Shows the biocompatibility of the prepared hydrogels.

4.7. Swelling studies:

This study shows the water absorbing capability of the prepared formulations.

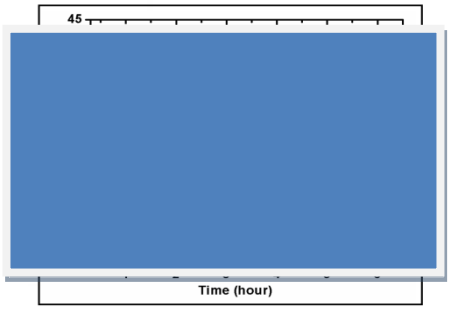

Figure 14 – Shows the swelling profile of the prepared hydrogels.

4.8. Drug release studies:

This study shows the release of the drug at pH 7.4.

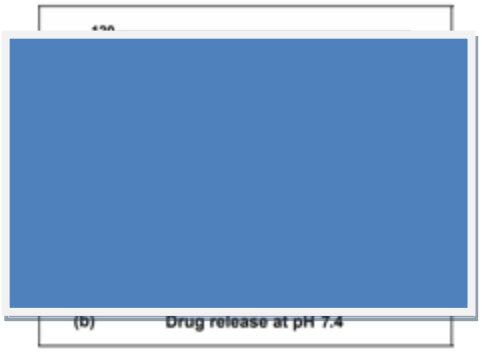

Figure 15 – Shows the drug release profile of the prepared drug loaded hydrogels.

4.9. Antimicrobial test:

This study is done to find the antimicrobial efficiency of the drug loaded hydrogels.

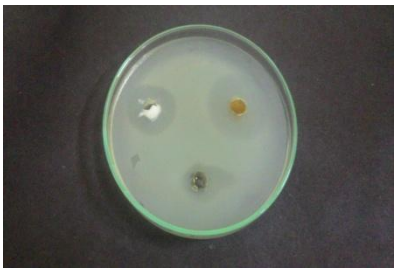

Figure 16 – Shows the antimicrobial activity of the prepared drug-loaded hydrogels.

CHAPTER – 4

CONCLUSION

- The current study explained the development of carrageenan and gelatin based hydrogels. These hydrogels were prepared by an easy and economical procedure. The developed gels were found to be smooth in texture, stable and hemocompatible in nature.
- The drug loaded gels showed sufficient antimicrobial efficacy so can be used as a topical antimicrobial gel.
- The carrageenan and gelatin based hydrogels could be tried for various biomedical applications, such as drug delivery systems and moist wound dressings.

CHAPTER – 5

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